Development of immuno-radiosensitizing nanosized zeolite for the treatment of glioblastoma

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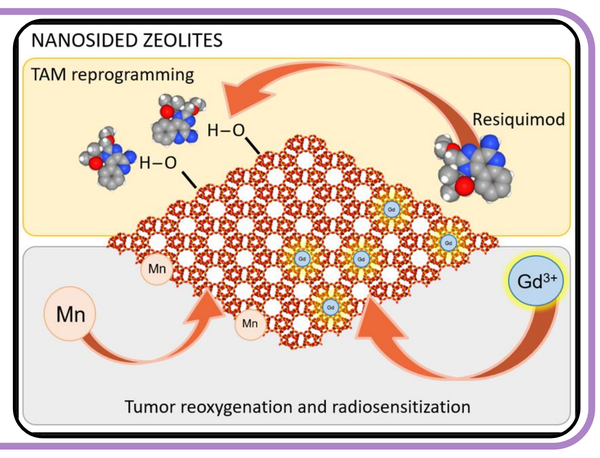
Context

Treatments for glioblastoma (GB) are not sufficiently effective. Its resistance to radiotherapy (RT) stems from intrinsic factors and severe tumor hypoxia, as RT efficacy depends on oxygen levels[1]. GB is a "cold" tumor microenvironment, infiltrated by pro-tumoral tumor-associated macrophages (TAMs) with an immunosuppressive and pro-tumor phenotype (M2-like).

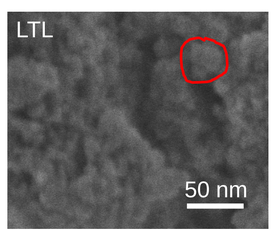
Strategies are explored to overcome these challenges : radiosensitizing the tumor with gadolinium (Gd)[2], reoxygenating it by *in situ* decomposition of H_2O_2 produced by cells and catalyzed by manganese (Mn)[3], and reprogramming TAMs to an anti-tumoral phenotype using TLR agonists like resiquimod (R848)[4].

In this study, the aim is to combine these strategies to develop a multimodal therapeutic nanoparticle (MTNP). This MTNP will be able to add both Gd and Mn, but also deliver TLR agonists in order to create, within a single entity, a synergy of radiosensitization, tumor reoxygenation, and TAM reprogramming.

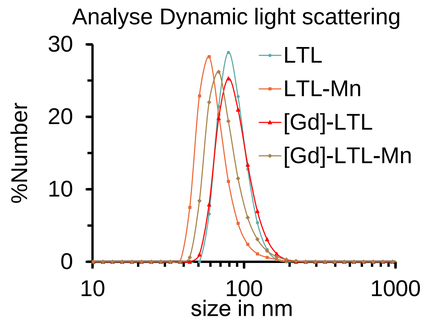


Characterization of zeolite nanoparticles

SEM image of LTL



Analyse Dynamic light scattering

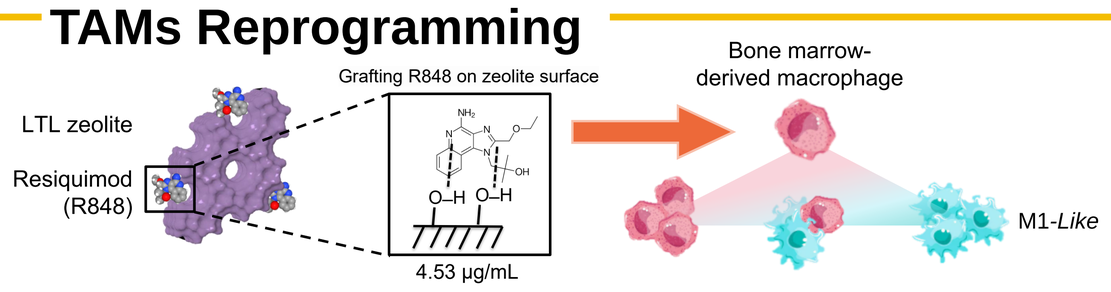


Elemental analysis by ICP of different zeolites

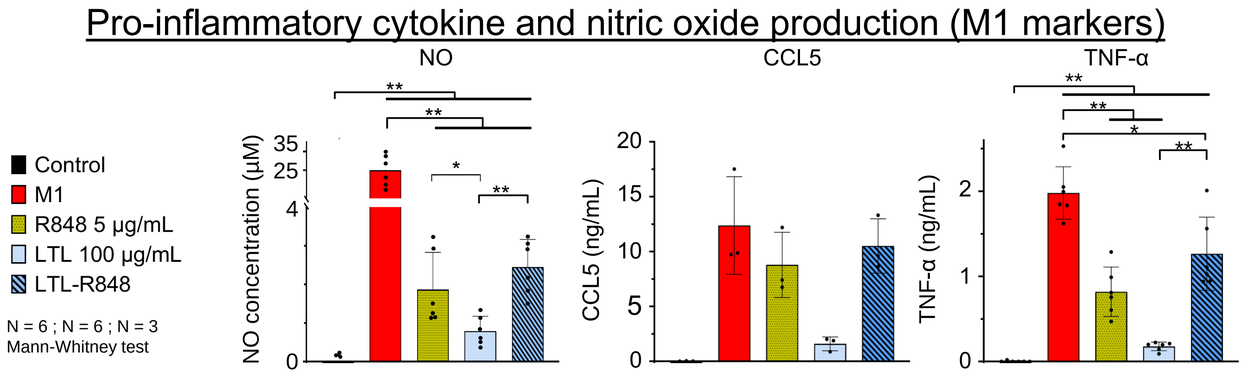
	55 Mn	157 Gd
LTL	0,00 %	0,00 %
LTL-Mn	1,92 %	0,00 %
[Gd]-LTL	0,01 %	7,49 %
[Gd]-LTL-Mn	1,27 %	1,97 %

→ **Both Gd and Mn were successfully incorporated into the LTL zeolite framework**

TAMs Reprogramming



Pro-inflammatory cytokine and nitric oxide production (M1 markers)



N = 6 ; N = 6 ; N = 3
Mann-Whitney test

→ **LTL-R848 treated macrophages are polarized toward a M1-Like phenotype**

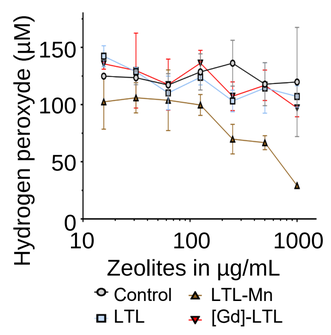
Oxygen production from H_2O_2

Manganese oxide grafting

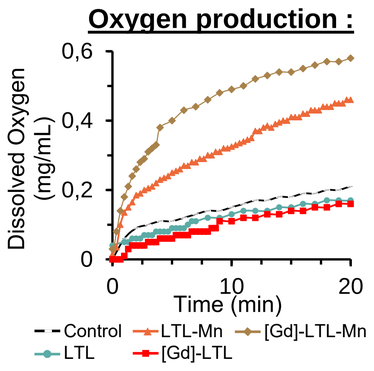
$H_2O_2 \xrightarrow{MnO_2} O_2 + H_2O$

→ **When zeolites are loaded with manganese oxides, they produce oxygen from H2O2**

Hydrogen peroxide dosage :

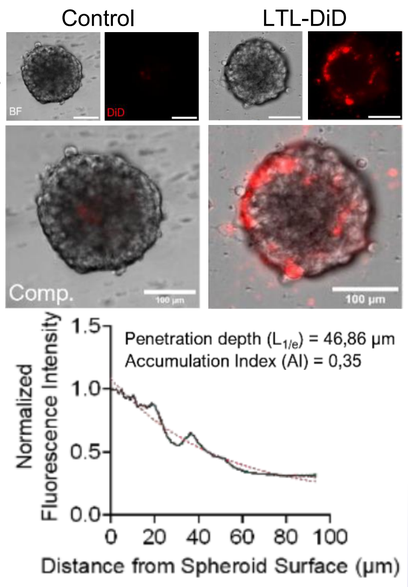


Oxygen production :



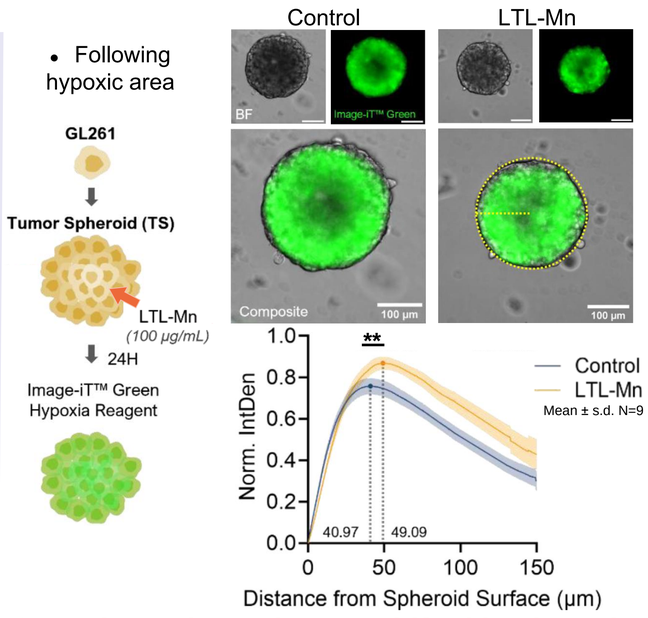
Reduction of the hypoxic zone in Tumor Spheroids (TS) :

- Following nanosized zeolites



→ **Zeolites remain on the periphery of the spheroids**

- Following hypoxic area

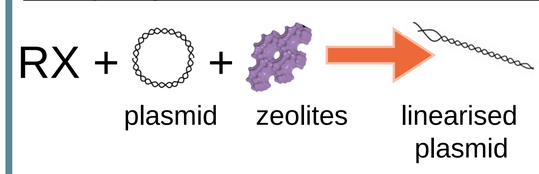


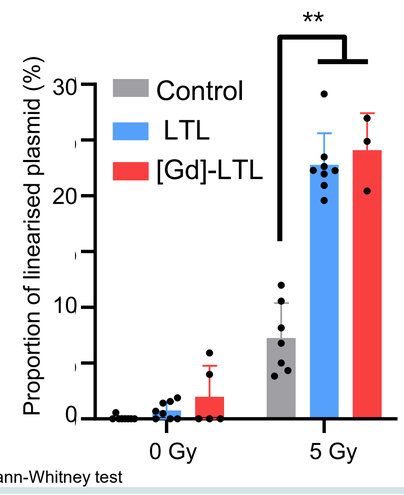
→ **Maximum hypoxic area shifted backwards along the path of manganese zeolites**

Radiosensitization

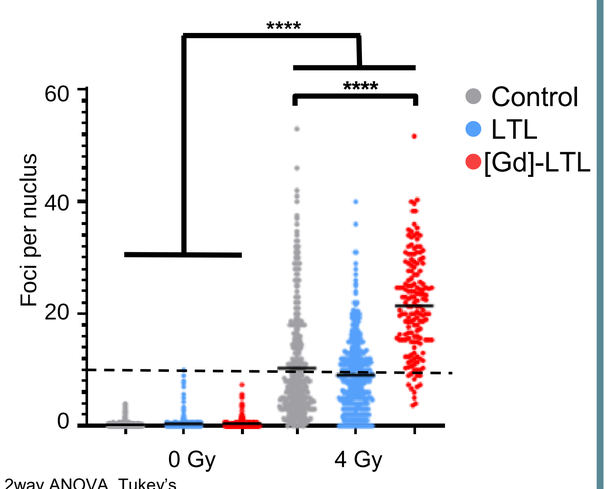
Radiation-matter interaction $RX + Gd^{3+} \rightarrow e^-$

Ability to generate double-strand breaks on DNA :

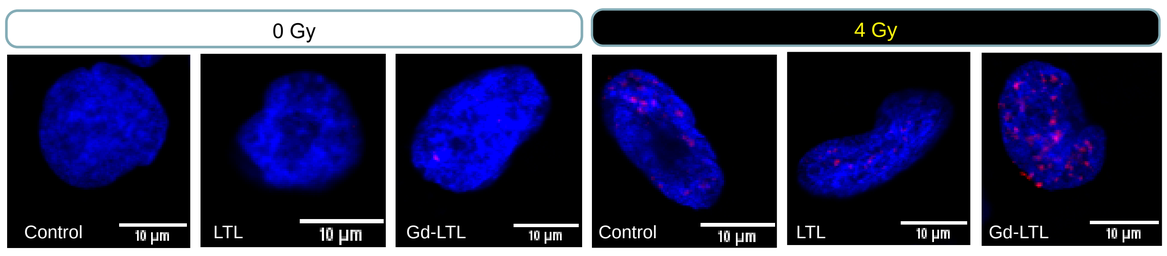




N = 3 ; Mann-Whitney test



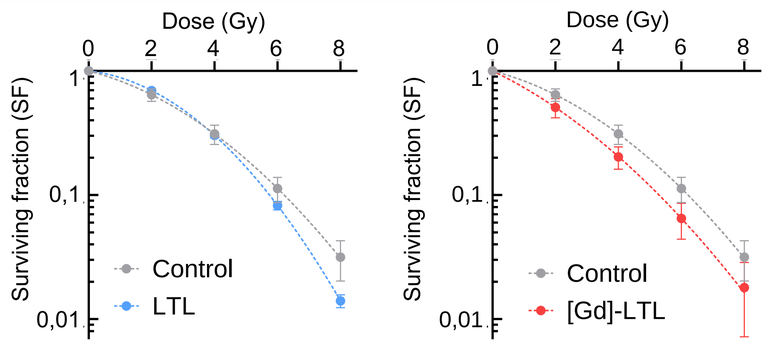
N = 3 ; 2way ANOVA, Tukey's



→ **Without RX, zeolites do not damage DNA**

→ **With RX, Gd-LTL have strong radiosensitizing properties**

Sensitisation of glioblastoma cells to X-rays :

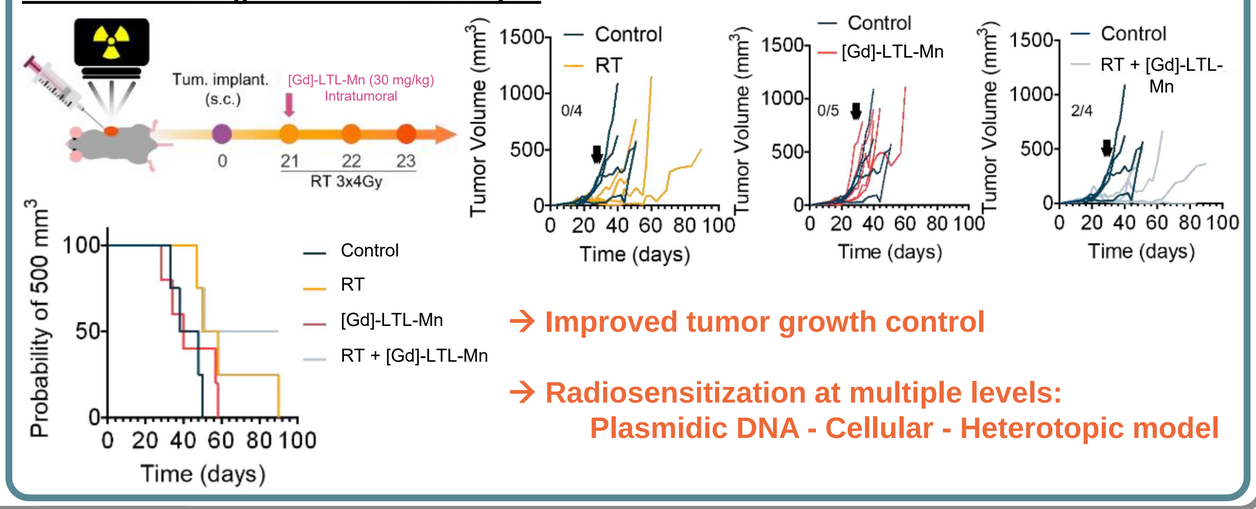


Parameter	α	β	Dose D_{37}
Control	0,1495	0,03565	3,56 ± 0,40 Gy ; N= 6
LTL	0,0638	0,05874	3,61 ± 0,08 Gy ; N= 2
[Gd]-LTL	0,2798	0,02941	2,73 ± 0,44 Gy ; N= 3

* Mann-Whitney test

→ **1 Gy shift of the mean lethal dose**

Sensitisation of glioblastoma to X-rays :



→ **Improved tumor growth control**

→ **Radiosensitization at multiple levels: Plasmidic DNA - Cellular - Heterotopic model**

Conclusion

These findings confirm the feasibility of combining multiple therapeutic approaches into a single MTNP.
However, further *in vitro* and *in vivo* studies are needed to confirm the synergistic potential of [Gd]-LTL-Mn with radiotherapy.
The reprogramming of TAMs should be thoroughly investigates before proceeding to *in vivo* models using the fully loaded MTNP ([Gd]-LTL-Mn: R848).

Acknowledgments

European Commission (Project : 101105382 — NanoImmunoRT — HORIZON-MSCA-2022-PF-01); CNRS MITI interdisciplinary programs; de l'Agence nationale de la Recherche (ANR) à travers le programme 'Investissements d'Avenir' (n°ANR-10-EQPX1401), French Foundation for Medical Research (grant no. FRM2024 ECO202406019136).

References :

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3. Prasad P *et al.* ACS Nano. 2014
4. Anfray C *et al.* J Immunother Cancer. 2021